

WHAT IS CLAIMED IS:

1                   1.       A method of preparing a cell culture of propagating pancreatic cells  
2       having the ability to be passed from one culture vessel to a second vessel at an initial  
3       concentration of about 180 cells per square centimeter and expanded to about 1,800 cells per  
4       square centimeter, while retaining the following properties: 90% PDX-1 positive and having  
5       an insulin:actin mRNA ratio of between 1:100 and 1000:1, said method comprising the steps  
6       of:

7                   (a) isolating propagating pancreatic cells;

8                   (b) transferring the cells to a culture medium containing growth hormone and  
9       having 1% or less total volume of serum to selectively propagate cells having an insulin:actin  
10      mRNA ratio of between 1:100 and 1000:1 and that are PDX-1 positive; and

11                  (c) passaging the cells to provide a cell culture of propagating pancreatic cells  
12      having the ability to be passed from one culture vessel to a second vessel at an initial  
13      concentration of about 180 cells per square centimeter and expanded to about 1,800 cells per  
14      square centimeter while retaining the following properties: 90% PDX-1 positive and having  
15      an insulin:actin mRNA ratio of between 1:100 and 1000:1.

1                   2.       The method of claim 1, wherein the cells are maintained in a first  
2       medium containing serum at between 1% and 4% of the total volume of the first medium  
3       before transfer to a second medium containing growth hormone and 1% or less total volume  
4       of serum.

1                   3.       The method of claim 1, wherein the cells are maintained in a first  
2       medium containing serum at above 4% of the total volume of the first medium before transfer  
3       to a second medium containing growth hormone and 1% or less total volume of serum.

1                   4.       The method of claim 3, wherein the cells are transferred from a first  
2       medium containing serum at above 4% of the total volume of the serum to a second medium  
3       containing growth hormone and less than 1% of total volume of serum by successive  
4       transfers to media having successively lower amounts of serum.

1                   5.       The method of claim 1, wherein the isolated propagating pancreatic  
2       cells have a mixture of PDX-1 positive and PDX-1 negative phenotypes.

1                   6.       The method of claim 1, wherein the cell culture has an insulin:actin  
2 mRNA ratio of between 1:10 and 100:1.

1                   7.       A method of producing an aggregate of cultured pancreatic cells that  
2 comprises an encapsulating mantle of ck-19 positive cells and an inner cell mass, wherein the  
3 aggregate comprises 50-5000 pancreatic cells and has a diameter of between 50 and 300  
4 microns, the method comprising the steps of

5                   (a) culturing pancreatic cells on a substrate;

6                   (b) removing the cells from the substrate;

7                   (c) reseeding PDX-1 positive pancreatic cells on a substrate produced by step  
8 (b); and

9                   (d) culturing the cells of step (c) on the substrate of step (c) to provide an  
10 aggregate of cultured pancreatic cells that comprise a surrounding mantle of ck-19 positive  
11 cells and an inner cell mass, wherein the aggregate comprises 50-5000 pancreatic cells and  
12 has a diameter of between 50 and 300 microns.

1                   8.       The method of claim 7, wherein the culturing of steps (a) or (c) takes  
2 place in a medium containing growth hormone and less than 1% total volume of serum.

1                   9.       A method of providing pancreatic endocrine function to a mammal, the  
2 method comprising the steps of

3                   (a) producing an aggregate of cultured pancreatic cells by the method of step  
4 7; and

5                   (b) implanting the aggregate within the mammal.

1                   10.     A method of claim 9 where the cells are passage in media containing  
2 between 1-2 mg per liter of recombinant growth hormone.

1                   11.     A method of claim 1 where the cells are passaged in media containing  
2 recombinant human growth hormone.

1                   12.     A method of claim 1 where the cells are passaged in media containing  
2 epithelial growth factor.

1                   13.     A culture of propagating pancreatic cells produced by the method of claim  
2 1.

1                   14.     A culture of propagating pancreatic cells having the ability to be  
2     passed from one culture vessel to a second vessel at an initial concentration of about 180 cells  
3     per square centimeter and expanded to about 1,800 cells per square centimeter while retaining  
4     the following properties: 90% PDX-1 positive and having an insulin:actin mRNA ratio of  
5     between 1:100 and 1000:1.

1                   15.     An aggregate of pancreatic cells produced by the method of claim 7.

2                   16.     An aggregate of cultured pancreatic cells, comprising an encapsulating  
3     mantle of CK19-positive cells and an inner cell mass, wherein the aggregate comprises 50-  
4     5000 pancreatic cells and has a diameter of between 50 and 300 microns.